UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JUL 29 2002

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Didecyl dimethyl ammonium chloride (DDAC); review of a 21-day dermal toxicity study in rats.

TO: Dennis Edwards, Chief
Velma Noble, Product Manager, Team 31
Regulatory Management Branch I
Antimicrobials Division (7510C)

FROM: Timothy F. McMahon, Senior Toxicologist 7/22/02
Antimicrobials Division (7510C)

THRU: Nader Elkassabany, Acting Team Leader NE 7/23/02
Team Two
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510C)

Norm Cook, Chief 07/29/02
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510C)

Registrant: The Procter & Gamble Company

DP Barcodes: D283093

Pesticide Chemical No.: 069149

A.I. Chemical Name: Didecyl dimethyl ammonium chloride (DDAC)

EPA File Symbol: 003573-00069

Action Requested:

On October 28, 1999 the U.S. EPA received a registration application for a new ready-to-use spray product called *Z-1 Fabric Refresher*, containing 0.13% of the registered antimicrobial chemical Didecyl dimethyl ammonium chloride (DDAC), proposed for a new use pattern as a bacteriostat against odor-causing bacteria on hard to launder fabrics. AD/RASSB was asked to conduct a residential non-dietary exposure and risk assessment in support of the proposed new use pattern for DDAC. This assessment was completed in the form of two memoranda (D260951, Residential non-dietary exposure assessment from Doreen Aviado, Biologist, and D260952, Toxicology review for DDAC from Jonathan Chen, Ph.D., Toxicologist). In these memoranda, non-dietary residential dermal risk was assessed using the available information on hazard and exposure for the DDAC active ingredient.

This assessment concluded that, based on the available data, a 21-day dermal toxicity study would be required as a condition of registration for the Z-1 product. The available data for calculation of the dermal MOEs, (which showed unacceptable risk for residential post-application scenarios) were based upon results of a repeated dermal toxicity study using the DDAC technical grade active ingredient (80.8% a.i.) and not the actual *Z-1* product (0.13% a.i.). It was felt at that time that, based upon previous Agency policy, a repeated dose dermal toxicity study with the actual Z-1 product would be needed in order to properly characterize risk from dermal exposure, as the systemic effects from exposure to the diluted product would occur at much higher doses than from exposure to the technical grade active ingredient, which would not be expected from residential use. However, it was also recognized that significant dermal irritation occurs even from exposure to diluted products containing DDAC as the active ingredient.

The registrant has conducted a 21-day dermal toxicity study with the DDAC active ingredient at the 0.13% concentration. The results of this study are presented below.

CITATION: Henwood, S.M. (2001) 21-Day Dermal Toxicity Study with SS0853.01 in Rats. Covance Laboratories, Inc. (Madison, Wisconsin). Study No. 6114-398, September 28, 2001. MRID 456566-01. Unpublished.

EXECUTIVE SUMMARY

In a 21-day dermal toxicity study (MRID 456566-01), SS0853.01 (100% pure) was administered directly to the skin of CD® [Crl:CD® (SD) IGS BR] rats (10/sex/group) at doses of 100, 500, and 1000 mg/kg-day. The dermal route of exposure was chosen because it is a possible route of human exposure. Doses for this study were determined by the Sponsor to achieve a gradient of toxic effects. The high-dose level was selected per OPPTS 870-3200 and was considered to show signs of toxicity. The mid-dose level was selected as an additional dose in order to evaluate any potential toxicological effects.

No treatment-related effects on clinical observations (including expanded clinical observations), motor activity, dermal irritation, ophthalmic observations, body weights or body weight changes, food consumption, clinical pathology parameters, terminal body weights, mean absolute or relative organ weights, or macroscopic or microscopic observations were observed. Analyses of hindlimb strength, food consumption, hematology and clinical chemistry parameters, and relative organ weights showed significantly reduced hindlimb strength in female rats at 500 and 1000 mg/kg/day; this effect was dose-related in females. Numerous microscopic changes in the liver were observed, but were noted to be test system-related due to the torso wrapping procedure.

Based on the reduced hindlimb grip strength observed in females at 500 mg/kg/day, the systemic NOAEL for SS0853-01 is 100 mg/kg-day in this study.

This 21-day dermal toxicity study is classified as **acceptable/guideline**, and satisfies the data requirement (OPPTS 870.3200) for a repeated dose [21/28-day] dermal toxicity study in rats.

DATA EVALUATION RECORD

21-DAY DERMAL TOXICITY STUDY WITH SS0853.01 IN RATS

Study Type: 21-Day Dermal Toxicity Study(Rat)

Prepared for

Antimicrobial Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

ICF Consulting Group
9300 Lee Highway
Fairfax, VA 22031

Under Subcontract to

Versar
6850 Versar Center
P.O. Box 1549
Springfield, VA 22151

Principal Reviewer	Kate Sullivan *Katherine Sullivan, M.S.*	Date 6/19/02
Independent Reviewer	Brenda Tondi *Brenda Tondi, Ph.D.*	Date 6/19/02
ICF Program Manager	Kara B. Altshuler *Kara Altshuler, Ph.D.*	Date 6/19/02
Versar Program Manager	_____ *Linda Phillips, Ph.D.*	Date _____

Contract Number: 68-W-01-036
Work Assignment No.: 0248.2000.002.02
EPA Work Assignment Manager: Cletis Mixon, Ph.D.

EPA Reviewer:Timothy F. McMahon, Ph.D. Date 7/22/02
Antimicrobials Division (7501C)

DATA EVALUATION RECORD

STUDY TYPE: 21/28-Day Dermal Toxicity Study - Rat; OPPTS 870.3200 (rodent).

DP BARCODE:D283093 SUBMISSION CODE: S614874
P.C. CODE: 069149

TEST MATERIAL (PURITY): SS0853.01 (100% pure)

SYNONYMS: None provided

CITATION: Henwood, S.M. (2001) 21-Day Dermal Toxicity Study with SS0853.01 in Rats. Covance Laboratories, Inc. (Madison, Wisconsin). Study No. 6114-398, September 28, 2001. MRID 456566-01. Unpublished.

SPONSOR: The Procter & Gamble Company
Cincinnati, OH

EXECUTIVE SUMMARY

In a 21-day dermal toxicity study (MRID 456566-01), SS0853.01 (100% pure) was administered directly to the skin of CD® [Crl:CD® (SD) IGS BR] rats (10/sex/group) at doses of 100, 500, and 1000 mg/kg-day. The dermal route of exposure was chosen because it is a possible route of human exposure. Doses for this study were determined by the Sponsor to achieve a gradient of toxic effects. The high-dose level was selected per OPPTS 870-3200 and was considered to show signs of toxicity. The mid-dose level was selected as an additional dose in order to evaluate any potential toxicological effects.

No treatment-related effects on clinical observations (including expanded clinical observations), motor activity, dermal irritation, ophthalmic observations, body weights or body weight changes, food consumption, clinical pathology parameters, terminal body weights, mean absolute or relative organ weights, or macroscopic or microscopic observations were observed. Analyses of hindlimb strength, food consumption, hematology and clinical chemistry parameters, and relative organ weights showed significantly reduced hindlimb strength in female rats at 500 and 1000 mg/kg/day; this effect was dose-related in females. Numerous microscopic changes in the liver were observed, but were noted to be test system-related due to the torso wrapping procedure. **Based on the reduced hindlimb grip strength observed in females at 500 mg/kg/day, the systemic NOAEL for SS0853-01 is 100 mg/kg-day in this study.**

This 21-day dermal toxicity study is classified as acceptable, with deficiencies in the study being discussed in the "Study Deficiencies" section of this evaluation report.

COMPLIANCE

Signed and dated GLP, Quality Assurance, and No Data Confidentiality Claims statements were included. According to those statements, the study was conducted according to GLP guidelines of the U.S. EPA and OECD. The quality assurance unit of the testing facility performed audits on the following study phases: test-material receipt, storage and handling, dose analysis, body weight, food consumption, animal observation, dose application and removal, expanded clinical observations, dose preparation, sample collection, necropsy, and data review. One protocol review, 2 report reviews, and 3 protocol amendment reviews also were conducted. A flagging statement was not included.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: SS0853.01
   Description: Clear liquid
   Lot/Batch #: Lot 0.01
   Purity: 100%
   CAS #: Not reported

2. Negative control: 20 negative control animals (10 per sex) were dosed with RO water (purified by reverse osmosis).

3. Test animals
   Species: Rat
   Strain: CD® [Crl:CD® (SD) IGS BR]
   Source: Charles River Laboratories, Portage, Michigan
   Acclimation period: 2 weeks
   Age and weight at study initiation: Animals were between 7 and 8.5 weeks old at study initiation. Body weight ranges for males and females at the start of dosing were 260-320 grams and 162-217 grams, respectively.
   Housing: Individually-housed in suspended, stainless steel cages.
   Diet: Certified Rodent Chow #8728C[1] was provided to all animals *ad libitum*
   Water: Tap water (municipal supply, City of Madison)[2] was provided to all animals *ad libitum*.

   Environmental conditions: Temperature: 19-25°C
   Humidity: 30-70%
   Air changes: Not reported

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[1] The study author indicated that the certification analysis of each feed lot was performed by the manufacturer. The feed reportedly contained no contaminants that were expected to interfere with the results of the study. Results of specified nutrient and contaminant analyses are reportedly maintained at Covance-Madison.

[2] The water supply in the laboratory is reportedly routinely analyzed for specified microorganisms and environmental contaminants. The results of the water analysis are maintained at Covance-Madison. The study author was not aware of any contaminants that were likely to be present in the water.

Photoperiod: 12 hour light/dark cycle

B. STUDY DESIGN

1. In life dates

Start: November 20, 2000 (study initiation)
End: September 28, 2001 (study termination)

2. Animal assignment

Animals were randomly assigned to control and treatment groups based on a computerized blocking procedure designed to achieve body weight balance with respect to the treatment group. The body weights of all rats placed on the study were within ±2 standard deviations of the mean body weight per sex. The number of animals per dose is summarized in Table 1 below.

Table 1. Study Design

Test Group	Exposure Concentration (mg/kg-day)[a]	Number of Animals	
		Male	Female
1	0	10	10
2	100	10	10
3	500	10	10
4	1000[b]	10	10

[a] Dosing volume was 1 mL/kg

[b]The high-dose group received the test material neat.

3. Dose Preparation and Analysis

A specified amount of vehicle (RO water) and the required volume of test article were placed into a labeled container, which was then mixed manually by inversion. Low- and mid-dose solutions were prepared independently in order of increasing concentration approximately every week. The high-dose article was prepared by placing the required volume of test article into a labeled container, since the high-dose group was administered neat. Dose preparations were stored at room temperature.

Homogeneity/Stability Analysis: According to the study author, homogeneity analyses were not required because dose levels selected for this study were in solution form. To test for stability, two sets of samples were taken from the low- and mid-dose preparations, mixed pretest, and analyzed. Samples were analyzed on the day of mixing and after being stored for at least 10 days at room temperature. The results of the stability analysis indicated that

after being stored for 10 days at room temperature, 100 and 99% of the initial dose (of 100 and 500 mg/kg-day doses, respectively) remained. Therefore, the dosing solutions were stable for 10 days at room temperature.

Concentration Analysis: Concentration analysis was performed every week by Covance using an analytical method, MP-PG98-MA. Concentrations were considered acceptable if the results of the individual or mean values were within 10% of the theoretical concentration. Stability samples analyzed from the low- and mid-dose were used for Week 1 routine analysis. All samples were stored at room temperature until analyzed. Data from Table 2 (pp. 54-56 of the study report) indicate that the actual dosing concentrations were within 94-99% of the theoretical concentrations and were acceptable for use in the study.

4. Dose Selection and Administration

Doses for this study were determined by the Sponsor to achieve a gradient of toxic effects. The high-dose level was selected per OPPTS 870-3200 and was considered to show signs of toxicity. The mid-dose level was selected as an additional dose in order to evaluate any potential toxicological effects.

Animals were exposed to the test article through dermal application. Approximately 10% of the total body surface area of the test animals was clipped 24-hours before the administration of the first dose. The exposure area was centered on the dorsal midline of the trunk of each animal and was marked with ink as needed. Skin abrading was avoided and maintenance clippings occurred approximately twice weekly or as needed thereafter.

Animals were exposed to the test article for at least 6 but no more than 7 hours/day for at least 21 days. The test article was applied directly to the skin of each test animal, spread uniformly with a syringe, covered with gauze dressing, and secured with nonirritating tape. A Vetwrap® elastic bandage was placed on top of the gauze dressing and was secured with Elastikon® tape. To prevent accidental ingestion of the test article, a flexible plastic collar was placed on all animals during the exposure period. Following the exposure period , all bindings and collars were removed and the exposure site was gently wiped with a disposable paper towel (moistened with RO water) to remove any control or test article.

5. Statistics

The statistical analyses are described in Table 2 below.

Table 2. Statistical analyses performed

Endpoints and Type of Analysis	
Endpoints	**Type of Analysis**
Body weights Body weight changes Food consumption Motor activity counts Grip strength Nociceptive reflex Continuous clinical pathology data Organ weight data	Levene's test was used to assess the homogeneity of group variances. A one-way analysis of variance (ANOVA) test was used if Levene's test was not found to be significant ($p>0.05$). If Levene's test was significant ($p\leq 0.05$), ANOVA was used on rank transformed data. Comparisons between control and treated groups were evaluated at the 5.0%, two-tailed probability level. *Post hoc* Dunnett's t-test was used to compare control and treated group means and incorporated transformation data when necessary.
Expanded clinical observation categorical data	Contingency Table methods were used.

C. METHODS

1. Observations

Animals were inspected twice daily for signs of morbidity, mortality, and signs of poor health and abnormal behavior. Once prior to treatment, on the day of initiation of treatment, and weekly, all animals were given clinical examinations that reported the following: changes in skin, fur, and mucous membranes; occurrences of secretions and excretions; changes in autonomic activity (i.e., lacrimation, piloerection, pupil size, or unusual respiratory pattern); changes in posture and reactivity to handling; and the presence of clonic or tonic movements, stereotypies (excessive grooming or circling), or bizarre behavior (e.g., self mutilation or walking backwards). Changes in gait were also measured.

Expanded clinical observations (hand-held and open field observations) were conducted on all animals once prior to treatment and weekly thereafter. Elicited behavior tests for sensory reactivity to stimuli and grip strength were evaluated once on Week 4 and 3, respectively.

During Week 4, motor activity was measured by placing each rat into an automated photocell activity recording device. Animals were monitored for 40-minute sessions, and activity counts were recorded at 2-minute intervals.

2. Body weight

Animals were weighed at receipt, at randomization, on the first day of treatment, and weekly thereafter.

3. Food consumption

Individual food consumption was determined weekly.

4. Ophthalmic examination

Ophthalmic examinations were performed by a board-certified veterinary ophthalmologist on all rats prior to treatment and on Day 19. Eyes were evaluated with an indirect ophthalmoscope and included the following: the anterior portion (eyelids, conjunctiva, cornea, anterior chamber, iris, and lens), the optic media (vitreous) and the ocular fundus.

5. Clinical Laboratory Tests

Blood was collected (via the jugular vein) from animals anaesthetized with an intraperitoneal injection of sodium pentobarbital prior to sacrifice for the analysis of hematology, coagulation, and clinical chemistry parameters as detailed below. The study author noted that animals were fasted prior to blood collection and were bled in random order.

a. Hematology

Hematology parameters determined in this study are indicated by an X in Table 3 below.[3] EDTA was added to all samples collected for hematology evaluations.

Table 3. Hematology parameters determined for animals with dermal exposure to SS0853.01 for 21 days

X	Hematocrit (HCT)*	X	Differential leukocyte count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB conc. (MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)*
X	Platelet count*	X	Blood Morphology
	Blood clotting measurements*		
X	Activated partial thromboplastin time		
X	Prothrombin time		

* Recommended for dermal exposure studies based on OPPTS Health Effects Test Guidelines 870.3200.

b. Clinical Chemistry

Clinical chemistry parameters determined in this study are indicated by an X in Table 4 below. No anticoagulant was used for clinical chemistry evaluations.

[3] A reticulocyte smear was also taken; however, it was not examined because there were no group differences observed in the erythrocyte or leukocyte measurements.

Table 4. Clinical chemistry parameters determined for animals with dermal exposure to SS0853.01

	ELECTROLYTES		OTHER
X	Calcium**	X	Albumin*
X	Chloride	X	Blood creatinine*
X	Phosphorus**	X	Blood urea nitrogen*
X	Potassium*	X	Total cholesterol*
X	Sodium*	X	Globulins
		X	Glucose*
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (ALK)*	X	Total serum protein (TP)*
X	Serum alanine aminotransferase (also SGPT)*	X	Albumin/Globulin (A/G) ratio
X	Serum aspartate aminotransferase (also SGOT)*	X	Triglycerides**
X	Gamma glutamyl transferase (GGT)*		

* Recommended for dermal exposure studies based on OPPTS Health Effects Test Guidelines 870.3200.
** Recommended for dermal exposure studies based on OPPTS Health Effects Test Guidelines 870.3200 if the test chemical is known or suspected of affecting related measures.

6. Urinalyses

Urine was collected on wet ice during week 4 (Day 24 or 25). Urinalysis parameters evaluated are included in Table 5 below.

Table 5. Urinalysis parameters determined for animals with dermal exposure to SS0853.01*

X	Appearance
X	Volume (about 16 hours)
X	Specific gravity
X	pH
X	Protein
X	Glucose
X	Blood
X	Ketones
X	Bilirubin
X	Urobilinogen
X	Microscopic examination of sediment

* Not required for dermal exposure studies based on OPPTS Health Effects Test Guidelines 870.3200

7. Sacrifice and Gross Pathology Examinations

At necropsy, all surviving animals were fasted overnight and bled from a jugular vein on Day 24 or 25. The animals were anesthetized with an intraperitoneal injection of sodium pentobarbital, weighed, exsanguinated, and necropsied in a random order. The carcass, external body orifices, the abdominal, thoracic, and cranial cavities, organs and tissues were examined for abnormalities.

8. Organ Weights

All organs indicated by an "XX" in Table 6 were weighed. Organ-to-body weight percentages and organ-to-brain weight ratios were calculated. Paired organs were weighed together.

9. Histopathology

All tissues (excluding the testes and epididymides) indicated by an "X" in Table 6 below were microscopically examined from the control and high-dose groups, and any animals that died prior to study termination. For microscopic evaluations, tissues were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined by a board-certified veterinary pathologist.

The testes and epididymides from each male animal sacrificed were separated. The testes were weighed together and the tunic of the testis was nicked to facilitate fixation in Bouin's fixative. After fixation, each testis was sectioned transversely in half, and the crania section was sectioned, embedded in paraffin, histologically processed, and stained with hematoxylin and eosin. The epididymides were weighed together, incised, and fixed in 10% neutral-buffered formalin. After fixation, the epididymides were sectioned longitudinally and processed for histologic evaluation. The remaining portions of the testicular tissue and epididymides were stored in 70% ethyl alcohol and 10% neutral-buffered formalin, respectively.

Table 6. Tissues weighed and microscopically evaluated for animals with dermal exposure to SS0853.01

X	DIGESTIVE SYSTEM	X	CARDIOVASC./ HEMAT.	X	NEUROLOGIC
X	Tongue	X	Aorta*	XX, X	Brain (3 levels)*+
X	Salivary glands (mandibular)*	XX, X	Heart*+	X	Periph. nerve (sciatic)*
		X	Bone marrow (femur, sternum)*	X	Spinal cord (3 levels)*
X	Esophagus*			XX, X	Pituitary*
X	Stomach (nonglandular & glandular)*	X	Lymph nodes (mandibular, mesenteric)*	X	Eyes (retina & optic nerve)*
					GLANDULAR
X	Duodenum*	XX, X	Spleen*+	XX, X	Adrenal glands*+
X	Jejunum*	XX, X	Thymus*+	X	Lacrimal gland (exorbital)
X	Ileum (including Peyer's patch)*			X	Mammary gland (female)*
			UROGENITAL	XX, X	Parathyroids*
X	Cecum*			XX, X	Thyroids*
X	Colon (proximal & distal)*	XX, X	Kidneys*+	X	Harderian gland
		X	Urinary bladder*		
X	Rectum*	XX, X	Testes*+		OTHER
XX, X	Liver*+	XX, X	Epididymides*+		
X	Urinary bladder	XX, X	Prostate*	X	Head
X	Pancreas*	X	Seminal vesicle(s)*	X	Bone (femur, sternum)
		XX, X	Ovaries*+	X	Skeletal muscle (thigh)
	RESPIRATORY	XX, X	Uterus (with uterine horns)*+	X	Skin* (treated and untreated)
				X	All gross lesions and masses*
X	Trachea*	X	Cervix		
X	Lung (with mainstem bronchi)*	X	Vagina		
X	Nose*				
X	Pharynx*				
X	Larynx*				

* Recommended for dermal exposure studies based on OPPTS Health Effects Test Guidelines 870.3200.
+ Organ weight required in dermal exposure studies based on OPPTS Health Effects Test Guidelines 870.3200.

II. RESULTS

A. Observations

1. Clinical Signs of Toxicity

Hand-held and open field observations in treated animals were comparable to controls. Mid- and high-dose females experienced a significantly decreased hindlimb strength; however, this finding was not considered treatment-related by the study author because the differences did not correlate with any other neurobehavioral parameters and was not present in males. There were no significant differences in motor activity testing values. Additionally, dermal irritation was limited to one mid-dose male that experienced very slight edema.

Table 7. Summary Table of Significant Clinical Findings

	Affected Females, Week 4 (n=10)			
Dose (mg/kg-day)	**0**	**100**	**500**	**1000**
Hind-limb grip strength	903±50.1	808±136.6	796±70.6[a,b]	780±109.1[a]

[a]p ≤0.05; [b]n=9

2. Mortality

No treatment-related mortality occurred during the study. One mid-dose female died of hepatopathy on Day 15. The study author noted that hepatopathy was probably caused by a mechanical injury of the liver due to the wrapping being too tight.

B. Body weight and weight gain

Body weights and body weight changes were similar in dosed and control animals.

C. Food consumption

Food consumption generally was similar in dosed and control animals. Low-dose females experienced a significant decrease in mean food consumption during Week 3; however, this finding was not considered to be treatment-related by the study author because it was not present at higher doses and similar effects were not observed in treated males.

D. Ophthalmoscopic examination

No treatment-related ophthalmic effects were observed.

E. Clinical Laboratory Tests

1. Hematology

No treatment-related differences were observed when evaluating hematology parameters. Mid-dose females experienced a statistically significant increase in absolute eosinophil count; our reviewers note that the value at the mid-dose was identical to that at the high dose. Therefore, the statistical significance is most likely due to the decreased number of females analyzed at this dose (n=9) compared to the other dose groups. This finding was not considered to be treatment related by the study author because it was not present at higher doses and a similar effect was not observed in treated males. Additionally, there were no corresponding pathology findings. Our reviewers agree with the study author's findings.

Table 8. Summary Table of Significant Hematology Findings

	Affected Animals (Week 4)							
	Males (n=10)				Females (n=10)			
Dose (mg/kg-day)	**0**	**100**	**500**	**1000**	**0**	**100**	**500**	**1000**
Mean eosinophil count ($x10^3$/mcL)	0.2± 0.07	0.2± 0.08	0.2± 0.07	0.2± 0.05	0.1± 0.06	0.2± 0.07	0.2± 0.08[a,b]	0.2± 0.08

[a] $p \leq 0.05$; [b]n=9

2. Clinical Chemistry

No treatment-related differences were observed when evaluating the clinical chemistry parameters. Low-dose females experienced a statistically significant increase in gamma glutamyltransferase (GGT); however, this finding was not considered to be treatment related by the study author because it was not present at higher doses and a similar effect was not observed in treated males. Additionally, there were no corresponding pathology findings. Our reviewers agree with the study author's conclusions.

Table 9. Summary Table of Significant Clinical Chemistry Findings

	Affected Animals (Week 4)							
	Males (n=10)				Females (n=10)			
Dose (mg/kg-day)	**0**	**100**	**500**	**100**	**0**	**100**	**500**	**1000**
GGT (IU/L)	2± 1.2	2± 0.8	2± 0.7	1± 0.7	2± 1.0	3± 0.5[a]	2± 0.9[b]	2± 1.4

[a] $p \leq 0.05$; [b]n=9

F. Urinalysis

No treatment-related differences were observed in the urinalysis data.

G. Organ weight

The study author noted that no treatment-related differences were observed when evaluating mean body weights or mean absolute or relative organ weights. Our reviewers noted that mid- and high-dose females experienced a statistically significant decrease in the kidney-to-brain weight ratio; however, this finding was not considered to be biologically significant because there was no corresponding pathology and a similar effect was not observed in treated males.

Table 10. Summary Table of Significant Organ Weight Findings

	Affected Animals							
	Males				Females			
Dose (ppm)	**0**	**100**	**500**	**1000**	**0**	**100**	**500**	**1000**
Kidney: Brain Weight	1.312± 0.099	1.345± 0.078	1.231± 0.206	1.293± 0.060	1.016± 0.078	0.962± 0.041	0.919± 0.061[a,b]	0.936± 0.094 [a]

[a] $p \leq 0.05$; [b] n=9.

H. Gross pathology

Gross pathological findings were unremarkable and sporadic and were not considered related to treatment by the study author or our reviewers.

I. Microscopic pathology

Numerous microscopic changes in the liver were observed and were noted by the study author to be test system-related, particularly the torso-wrapping procedure (Parker, 1995), and not test article-related. The observed hepatopathy was characterized by focal to mutifocal hepatocellular necrosis and loss with capsular/subcapsular fibrosis and irregularity, accompanied by variable cellular infiltrates, including pigmented macrophages. Frequently, these changes were observed in one of the liver lobes, while the other remained unaffected. Our reviewers also note that there was a high incidence of mineralization in the kidneys for both control and treated females. This observation was not considered to be related to the test article since a similar incidence rate was observed between control and high-dose females, and male animals did not experience a similar effect. Our reviewers note that male and female rats at the 100 mg/kg-day dose and males at the 500 mg/kg-day dose were not evaluated for gross pathology; one female at the 500 mg/kg-day dose was evaluated for gross pathology.

Table 11. Summary Table of Significant Gross Pathology Findings

	Affected Animals							
	Males				Females			
Dose (mg/kg-day)	**0**	**100**	**500**	**1000**	**0**	**100**	**500**	**1000**
Kidney								
Mineralization	1/10[a]	0/0	0/0	0/10	6/10	0/0	1/1	6/9
Liver								
Infiltrate, lymphohistiocytic	10/10	0/0	0/0	10/10	9/10	0/0	1/1	7/9
Infiltrate, pigmented macrophage	2/10	0/0	0/0	4/10	3/10	0/0	0/1	2/9
Fibrosis	2/10	0/0	0/0	6/10	3/10	0/0	1/1	3/9
Hepatocellular degeneration	0/10	0/0	0/0	1/10	0/10	0/0	1/1	1/9
Hepatopathy	4/10	0/0	0/0	7/10	3/10	0/0	0/1	3/9
Hepatocellular necrosis	2/10	0/0	0/0	3/10	0/10	0/0	1/1	0/10

[a]Number of animals examined

III. DISCUSSION

A. The study author noted that there were no treatment-related effects on clinical observations (including expanded clinical observations), motor activity, dermal irritation, ophthalmic observations, body weights or body weight changes, food consumption, clinical pathology parameters, terminal body weights, mean absolute or relative organ weights, macroscopic or microscopic observations. Based on these results, the study author concluded that the NOEL for this study was 1000 ppm for both males and females.

Although there were no other apparent neurologic abnormalities observed, the cause of the decreased hindlimb grip strength in female rats at the 500 and 1000 mg/kg/day dose levels was not specifically investigated. This effect was statistically significant, was dose-related, and is relevant to the action of chemicals in the quaternary ammonium class, which have been used historically as ganglionic blocking agents in pharmacology. Therefore, this should be considered an adverse effect in this study until proven otherwise.

Based on the decreased hindlimb grip strength observed in female rats at 500 mg/kg/day, the systemic NOAEL in this study is determined to be 100 mg/kg/day.

B. Study deficiencies

Overall, our reviewers note that the study design and conduct appeared adequate for the stated purpose. The only noted deficiency was the omission of the gallbladder from histopathological examination; this organ is a recommended tissue for microscopic evaluation. This deficiency is not expected to alter the outcome or the conclusions of the study.

IV. REFERENCES

Parker GA and Gibson WB. Liver lesions in rats associated with wrapping of the torso. Tox Pathol 1995; 23:507-512.

APPENDIX A: DATA VALIDATION

21-Day Dermal Toxicity Study with SS0853.01 in Rats

I. ANIMALS GROUPS FOLLOWED THROUGHOUT THE STUDY

Data from all rat groups were reviewed in the study report, including the summary data and raw data included in the appendices. Four groups of 10 rats/sex were followed throughout the study report, at concentrations of 0, 100, 500, or 1,000 mg/kg-day and a dose volume of 1 mL/kg.

II. CRITICAL EFFECTS AND SPOT CHECKS

Incidence counts or means and standard deviations of the means were calculated as appropriate using the individual animal data provided in the appendices of the study. The results were compared to corresponding summary tables in the study report. For each table reviewed, different data items were selected at random to provide "spot check" validations of the information presented in the tables.

Table 1 (p. 52-53): Results of Stability Analysis; all data.

Table 2 (p. 54-56): Results of Dose Preparation Analyses (mg/mL); all data

Table 3 (p. 57): Summary of Clinical Observations; all data
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Table 4 (p. 58-59): Summary of Expanded Clinical Observations Predose; all data

Table 5 (p. 60-61): Summary of Expanded Clinical Observations Week 1; all data

Table 6 (p. 62-63): Summary of Expanded Clinical Observations Week 2; all data

Table 7 (p. 64-65): Summary of Expanded Clinical Observations Week 3; all data

Table 8 (p. 66-68): Summary of Expanded Clinical Observations Week 4; all data

Table 9 (p. 69): Summary of Expanded Clinical Observations Grip Strength Data (g)-Week 4; group 1 and group 4

Table 10 (p. 70): Summary of Expanded Clinical Observations Nociceptive Reflex Data (seconds)-week 4; all data

Table 11 (p. 71-72): Summary of Motor Activity Data-males; groups 0 and 1,000 mg/kg-day

Table 12 (p. 73): Summary of Ophthalmic Observations; all data

Table 13 (p. 74): Summary of Dermal Irritation Data; all data
Table 14 (p. 75): Summary of Body Weight Data (g); week 2 and week 4

Table 15 (p. 76): Summary of Body Weight Change Data (g); week 1 to 2 and week 1 to 4

Table 16 (p. 77): Summary of Food Consumption Data (g); all data

Table 17 (p. 78-81): Summary of Clinical Hematology Data; dose groups 100 and 1,000 mg/kg-day

Table 18 (p. 82-85): Summary of Clinical Chemistry Data; dose groups 0 and 500 mg/kg-day

Table 19 (p. 86-87): Summary of Clinical Urinalysis Data; all data

Table 20 (p. 88-101): Summary of Organ Weight Data;

Male- Adrenal (groups 1 and 3), Brain (groups 2 and 4), Epididymides (groups 1 and 3), Heart (groups 2 and 4), Kidney (groups 1 and 3), Liver (groups 2 and 4), Pituitary (groups 2 and 4), Prostate (groups 1 and 3), Spleen (groups 2 and 4), Testis (groups 1 and 3), Thymus (groups 2 and 4), Thyroid/Parathyroid (groups 1 and 3),

Female: Adrenal (groups 2 and 4), Brain (groups 1 and 3), Heart (groups 1 and 3), Kidney (groups 2 and 4), Liver (groups 1 and 3), Ovary (groups 2 and 4), Pituitary (groups 1 and 3), Spleen (groups 1 and 3), Thymus (groups 1 and 3), Thyroid/Parathyroid (groups 2 and 4), Uterus (groups 1 and 3)

Table 21 (p. 102-110): Incidence of Macroscopic Observations; male groups 2 and 4, female groups 1 and 3

Table 22 (p. 111-120): Incidence of Microscopic Observations; male groups 1 and 3, female groups 2 and 4

III. FINDINGS

Any inconsistencies between values listed in the study report and means, standard deviations, and incidence counts determined by the data validator do not appear to be significant. There were no findings from this review that are expected to substantially affect the study results.

Liana Grossman

Liana Grossman, B.A.

06/20/02

Date